Virtual Cell Version 4.0 Tutorial I

Creating a FRAP BioModel

Introduction

Fluorescence Recovery After Photobleaching (FRAP) is a fluorescent optical technique used to measure the dynamics of a molecule over time and chemical changes of molecular species. The fluorescently labeled molecules are visualized through an epifluorescent or confocal microscope using low light excitation. The excitation light is focused onto a small region of molecules and pulsed to a high intensity in order to photobleach the fluorophore within the illuminated region. A blackened area of photobleached molecules surrounded by fluorescently labeled molecules that are not photobleached will result from the high intensity light. The molecules that are not photobleached will diffuse, providing they can, into this region. The blackened area will gradually increase in intensity over time as the molecules diffuse in.

The percent recovery and diffusional mobility can be determined from FRAP experiments. Percent recovery tells how much light returns to the area as compared to the amount of light before photobleaching. Diffusional mobility is the measurement of how quickly the fluorescent molecules migrate into the photobleached region. Fluorescent recovery usually does not reach 100%.

Following the Tutorial

You can create your own BioModel and Application as you read through the tutorial or you may choose to load the public version of this model. Go to View>Private Only and make sure this option is deselected so you will be able to view the public model in the Tutorial folder. The public BioModel is Tutorial_FRAP, the Application is FRAP, and the simulation results are saved as FRAP1.

## Defining the biological model

Creating and Defining Compartments

The biological model is defined as a collection of biochemical reactions acting on a set of molecular species localized in specific cellular structures. The cellular structures are defined as mutually exclusive compartments within the cells, as well as membranes that separate them. The compartments represent three-dimensional volumetric regions while the membranes represent two-dimensional surfaces separating the compartments. All structures can contain molecular species and a collection of reactions that describe the biochemical behavior of those species within that structure. Keep in mind when developing your model that it must be mapped to a specific cellular geometry before any quantitative simulation or analysis can be performed.

When the software is initiated, you are presented with a BioModel document. Select the compartment once with the left mouse button, the region will turn red. Use the right mouse button to access the Properties menu. Enter the name "EC", for extracellular, in the Feature Name text field and press OK.

Select the compartment tool once and click in the extracellular compartment. The New Feature Dialog will appear. Type in "Cell" in the Feature Name text field, and "PM" (PlasmaMembrane) in the Membrane Name text field. Press Add Feature to create the new compartment and membrane.

Adding Species
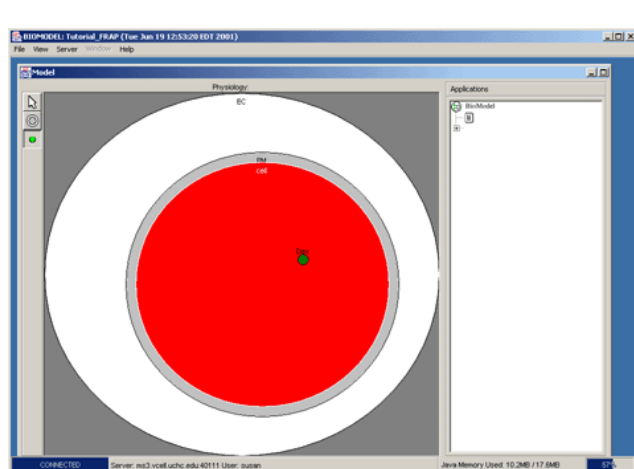
Select the species tool and click once in the Cell compartment. The Add New Species dialog will appear. Type "Dex" in the Name text field. Press Annotate and type in "Dextran"; press OK. Press Add to create the new species.
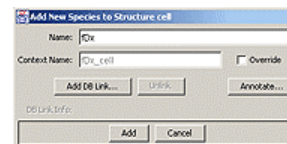
The BioModel is complete and you should have a model that looks similar to the image. Save the model at this point before proceeding further. Go to File>Save As and enter a unique name in the text field, press OK.

The FRAP Application

Introduction

Each model being developed requires an Application, which consists of a detailed description of the cellular geometry, Structure Mapping, Initial Conditions, and Reaction Mapping. The geometry represents the morphometry of a particular cell or portion of a cell. The geometry may be captured by various imaging modalities such as wide field, confocal, or electron microscopy. Analytic geometry may be used to define very regular structures or symmetric cells.
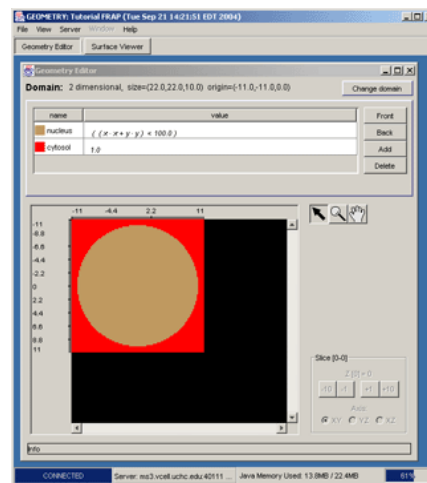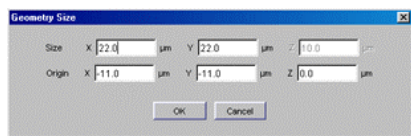
Creating the Geometry

Spatial Geometry

In the BioModel document, go to File>New>Geometry>Analytic>2-D. The Geometry document will open with a single compartment automatically created in the Geometry Editor. Press Add one more time to create an additional subVolume. The two subVolumes you create will represent the extracellular and cytosolic compartments.

Double click the name text field for one of the subVolumes; type in "Cell" and press Enter. Double click the value text field; enter the following equation to define a circle: ((x*x+y*y)<100.0) If the circle you have just defined is not visible, select the Cellvolume and press Front to bring it in front of the extracellular volume.

Double click the name text field for the other subVolume. Type in "EC" and press Enter. Leave the value at 1.0.

Press Change domain to access the Geometry size dialog. Enter "22" in the X and Y size text fields, and enter "-11" in the X and Y origin text fields. Press OK to accept the values and to close the window.

Save the geometry by going to File>Save.

Creating the Application

Structure Mapping

Change from the Geometry document back to the BioModel document. Go to the Application panel of the document, press Application>New and supply an Application name. The Application will default to the Structure Mapping tab with a compartmental model mapped. Press the View/Change Geometry button at the bottom and select the geometry you just created from the Geometry database. The Geometry will appear in the Structure Mapping tab.

Use the line tool to map the physiology model to the geometric representation. Map EC to EC and cell to cell. You need to reselect the line tool each time you do mapping, and you need to map from the physiology to the Geometry.

Initial Conditions

Select the Initial Conditions tab. Double click the Initial Conditions text field and type in the following equation in the Expression Editor text box:

$$(10.0*((x<-5.0)||(x>5.0)||(y<-5.0)||(y>5.0)))$$

Press OK to accept the equation and to close the window. This equation defines the initial bleached region and/or it defines where the initial concentration of the fluorescent dextran is located. Enter 20 in the Diffusion Rate text field and press Set to accept the value.
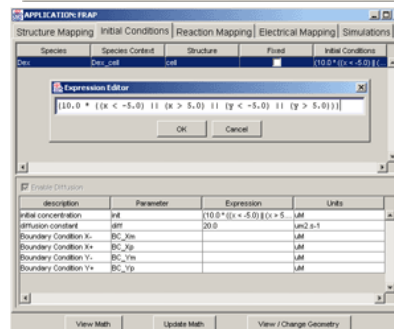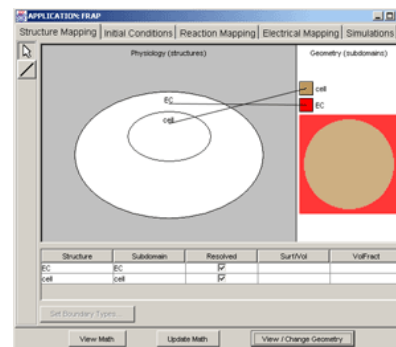
Note that Reaction Mapping does not apply to this model since there aren't any reactions described. Resave your model before proceeding to the simulation.

## Running a FRAP Simulation and Generating Results

Simulations

Select the Simulation tab and press the New button in the Simulations dialog.

In the upper panel of the Simulations dialog, a simulation with a default name will appear. Double click the Simulation Name text field, and enter a name. You may also enter information about the simulation in the Comments text field.
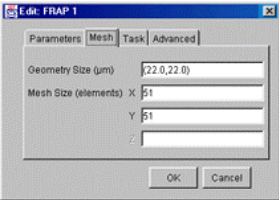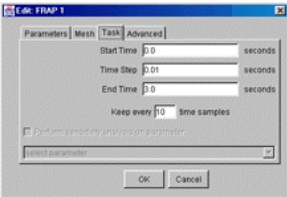

Make sure the Simulation name is selected; press Edit to access the additional runtime features: Parameters, Mesh, Task and Advanced.
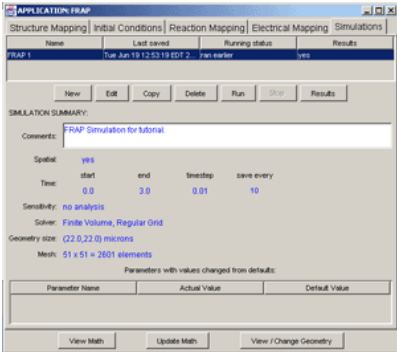
Select the Mesh tab. Enter "51" for the X and Y dimensions for the Mesh Size. The Geometry Size should be listed as (22.0,22.0, 0).

Select the Task tab to set the run time parameters.

Start Time: 0
Time Step: .01
End Time: 3.0
Save Interval: 10

Press OK to accept all the entries and to close   the Edit dialog.

Select the simulation and press Run to initiate   the simulation. Your model will automatically be saved with the run   conditions and the simulation will begin. Once results have been   generated, press the Results button to see them. You may have   to reselect the Simulation in order to activate the Results   button. Use the scroll bar on the left side of the results dialog to   change the time interval or enter a time in the Time text field and   press Enter.

You can display the results in either a Gray or a   Blue-Red color map. You can also toggle between auto and   manual scaling. Enter values in the Min and Max text fields for   manual scaling. Remember to press Enter to accept the value   and to update the image display.
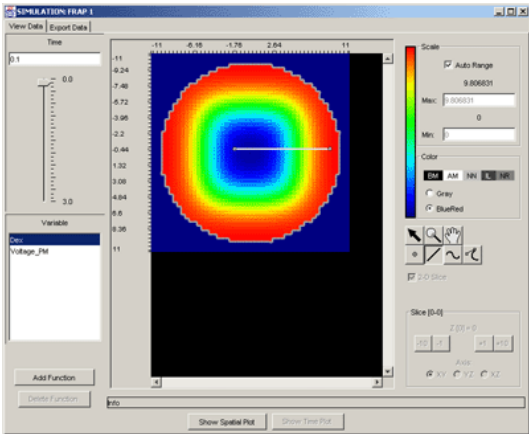
Use the Line or Spline tools to define regions of interest for a Spatial plot. Press Spatial plot to view the graph.
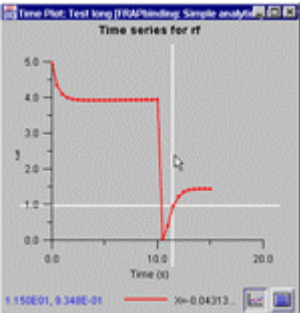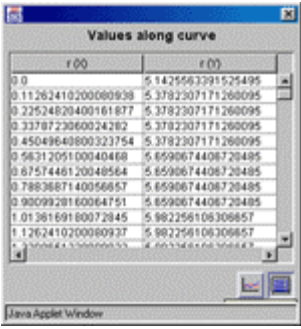

Use the Point tool to define a point for a Time plot. Press Time Plot to view the graph.

Hold down the right mouse button, while over the graph, to display the Plot Settings dialog.

Select your options for Auto Scale, Stretch, Draw nodes, Show crosshairs, and Snap to nodes. Press OK to accept your options and to close the dialog.
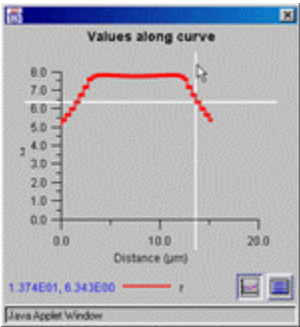
You can also view the data values by pressing the Show Values icon. You can copy the values directly into a tab delimited spreadsheet. Use ctrl C to copy individual cells and ctrl K to copy all the data values.

Export Features

Press Show export controls to access the export dialog. Depending on your model, you can export data from a single time point or range of time points, and a single variable or several variables, in a variety of different formats. Please see chapter 9 of the User Guide, Exporting Simulation Results, for additional information.